Short Communications

The isolation of cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone) from a saltant of *Helminthosporium victoriae*

During the course of the isolation of the toxin of *Helminthosporium victoriae*[1], it was often noted that cultures of the fungus grown on Richard's medium lost their ability to produce toxin and red-colored saltants appeared. This red-colored variation was also found in stock cultures grown on oatmeal agar and stored at 5°. No direct correlation between loss of toxin and production of pigment was established. Many inactive saltants were obtained that showed no red color.

The red-colored mycelium was washed a number of times with distilled water, pressed free of liquid on a mechanical press, and dried in a hot air oven at 60°. The dry mycelium (16.8 g) was mixed with quartz sand and ground in a mortar. After a short preliminary extraction with petroleum ether, the pigment was extracted by chloroform. Continuous extraction for five days in a Soxhlet apparatus was necessary to remove the bulk of the pigment from the mycelium. The chloroform extract was evaporated to a small volume and, on cooling in an ice bath, crystallization took place. On removing the crystals by filtration and washing with cold chloroform, bronze platelets were obtained which melted* with sublimation at 230–265°. This impure material was recrystallized from pyridine, followed by recrystallization from chloroform. The material was finally sublimed under high vacuum at 175°, producing fine red-bronze crystals which melted at 274.5–275.0°. A total yield of 170 mg of crystalline pigment was obtained, equivalent to about 1% of the dry weight of the mycelium.

Found** C = 63.13%; H = 3.59%
Calculated for $C_{15}H_{10}O_6$ C = 62.93%; H = 3.51%

An acetyl derivative of the pigment was prepared using acetic anhydride and sulfuric acid. After crystallization from acetic acid and recrystallization from ethanol, fine lemon-yellow needles were obtained which melted at 232°.

Found C = 61.01%; H = 3.98%
Calcd. for tetraacetyl derivative $C_{23}H_{18}O_{10}$ C = 60.79%; H = 3.96%

The red pigment thus analyzed well for a tetrahydroxyanthraquinone derivative. Among four such compounds isolated by RAISTRICK, the melting points agreed best with cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone)[2] (260–261°) and its tetraacetate (224–226°). Like this compound, the isolated pigment was insoluble in aq. Na_2CO_3. Furthermore, the acetyl derivative like that of cynodontin was resistant to oxidation by CrO_3 in acetic acid and acetic anhydride.

To confirm the identity, 1,4,5,8-tetrahydroxy-2-methylanthraquinone was synthesized according to the procedure of ANSLOW AND RAISTRICK[3]. The purified synthetic material melted at 272.5–273.0° and caused no depression of the melting point of the isolated pigment. In addition, a tetraacetyl derivative of the synthetic material was found to melt at the same point as the natural derivative and no depression of this melting point was observed when samples of the synthetic and natural derivatives were mixed. Furthermore, the spectrum of the synthetic material in the ultraviolet, visible and infrared regions was found to be coincident with that of the isolated material***.

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[1] R. B. PRINGLE AND A. C. BRAUN, *Phytopathology*, 47 (1957) 369.
[2] H. RAISTRICK, R. ROBINSON AND A. R. TODD, *Biochem. J.*, 27 (1933) 1170.
[3] W. K. ANSLOW AND H. RAISTRICK, *Biochem. J.*, 34 (1940) 1546.

* Melting points were obtained on a Fisher-Johns micro hot-stage apparatus.
** Microanalyses by Mr. T. BELLA of the Rockefeller Institute.
*** Infrared spectra were compared by Dr. H. JAFFE of the Rockefeller Institute.

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